Slc7a11 gene controls production of pheomelanin pigment and proliferation of cultured cells

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In mammals, >100 genes regulate pigmentation by means of a wide variety of developmental, cellular, and enzymatic mechanisms. Nevertheless, genes that directly regulate pheomelanin production have not been described. Here, we demonstrate that the subtle gray (*sut*) mouse pigmentation mutant arose by means of a mutation in the *Slc7a11* gene, encoding the plasma membrane cystine/glutamate exchanger xCT [Kanai, Y. & Endou, H. (2001) *Curr. Drug Metab.* 2, 339–354]. A resulting low rate of extracellular cystine transport into *sut* melanocytes reduces pheomelanin production. We show that *Slc7a11* is a major genetic regulator of pheomelanin pigment in hair and melanocytes, with minimal or no effects on eumelanin. Furthermore, transport of cystine by xCT is critical for normal proliferation, glutathione production, and protection from oxidative stress in cultured cells. Thus, we have found that the *Slc7a11* gene controls the production of pheomelanin pigment directly. Cells from *sut* mice provide a model for oxidative stress-related diseases and their therapies.

glutathione | melanin | pigmentation | cystine | melanocyte

Pheomelanin (red/yellow) pigment is produced by the addition of cysteine to dopaquinone (1). Both pheomelanin and eumelanin (brown/black) pigments protect skin from UV damage. However, pheomelanin also serves an opposing role as a potent UV photosensitizer, possibly contributing to increased sensitivity of fair-skinned individuals with yellow or red hair to sunburn, premature aging, and/or malignant transformation (2). In mice and other mammals, pheomelanin in the typical agouti-banding pattern provides camouflage, and in mammals and birds, pheomelanin pigmentation patterns are important components of the mechanisms of sexual recognition and display (3).

It has been suggested, although not proven, that the recessive subtle gray (*sut*) mouse pigmentary mutation reduces yellow pigmentation (4). *sut* also has moderate deficiencies of electron microscopically observable platelet-dense granules, qualifying it as a model (5) for a mild form of Hermansky–Pudlak syndrome (HPS), a genetically heterogeneous inherited disease characterized by abnormalities in biosynthesis and/or trafficking of lysosome-related organelles, including melanosomes, platelet-dense granules, and lysosomes (6, 7). In mice, at least 16 models of HPS are known (8). Several HPS genes encode proteins with known functions in vesicle trafficking such as subunits of the AP-3 adaptor protein complex, the Rab geranylgeranyltransferase complex, and the class C protein complex. However, the majority are genes of unknown function and are found only in higher metazoans (8).

Materials and Methods

Mice and Genetic Crosses. Mutant *sut* and control C3H/HeSnJ mice were obtained from The Jackson Laboratory and subsequently bred in the animal facilities of Roswell Park Cancer Institute. All procedures (mouse protocols 125M) were approved by the Roswell Park Institutional Animal Care and Use Committee and adhered to the principles of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Identification of the *sut* Gene. High-resolution genetic and physical maps of the *sut* critical region were generated with a backcross between homozygous *sut/sut* mice and PWK wild-type mice (subspecies *Mus musculus musculus*) as described in ref. 9 and based on the National Center for Biotechnology Information map viewer (build 32.1). Altogether, we typed 1,474 backcross progeny at 6 weeks of age for the *sut* pigment phenotype and for crossovers in the region surrounding *sut* by using the flanking microsatellites *D3Mit3* (proximal) and *D3Mit153* (distal).

We used 3′ RACE of mouse brain mRNA to find the alternative 3′end of the *Slc7a11* mRNA in *sut* brain (GenBank accession no. AY766237). Two alternative transcripts in C3H/HeSnJ brain cDNA with different poly(A) signals (GenBank accession no. AY766236) were identified.

Expression Analysis. Total RNA was reverse transcribed as described in ref. 9. For Northern blot analysis of *sut* transcripts, poly(A)-mRNA (4 μg from brain and 2 μg from melanocytes) was isolated according to Promega PolyATract kit instructions, blotted, and hybridized with a transcript-specific 300-bp *sut* ^{32}P-radiolabeled probe (exons 9–11).

Cell Culture. The *sut* gene was transferred from the C3H/HeSnJ strain to the C57BL/6J strain by backcrossing for six generations. Melanocyte lines from *sut/sut* mutants were generated rapidly by deriving cell cultures carrying the *Ink4a–Arf* deletion to prevent cell senescence, as described in ref. 10. The C57BL/6J melan-a cell line (11) was used as wild-type control. Fibroblasts from skin of newborn C3H/HeSnJ and *sut* were established as described in ref. 12. Thioglycollate-elicited mouse peritoneal macrophages were isolated and cultured as described in ref. 13.

Cell numbers were measured in a Coulter Counter after trypsinization. Trypan blue assays indicated >95% viability of *sut* melanocytes after 1–4 days culture with β-mercaptoethanol (βME), whereas >90% were nonviable after 4 days culture without βME.

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Abbreviations: HPS, Hermansky–Pudlak syndrome; βME, β-mercaptoethanol; BAC, bacterial artificial chromosome.

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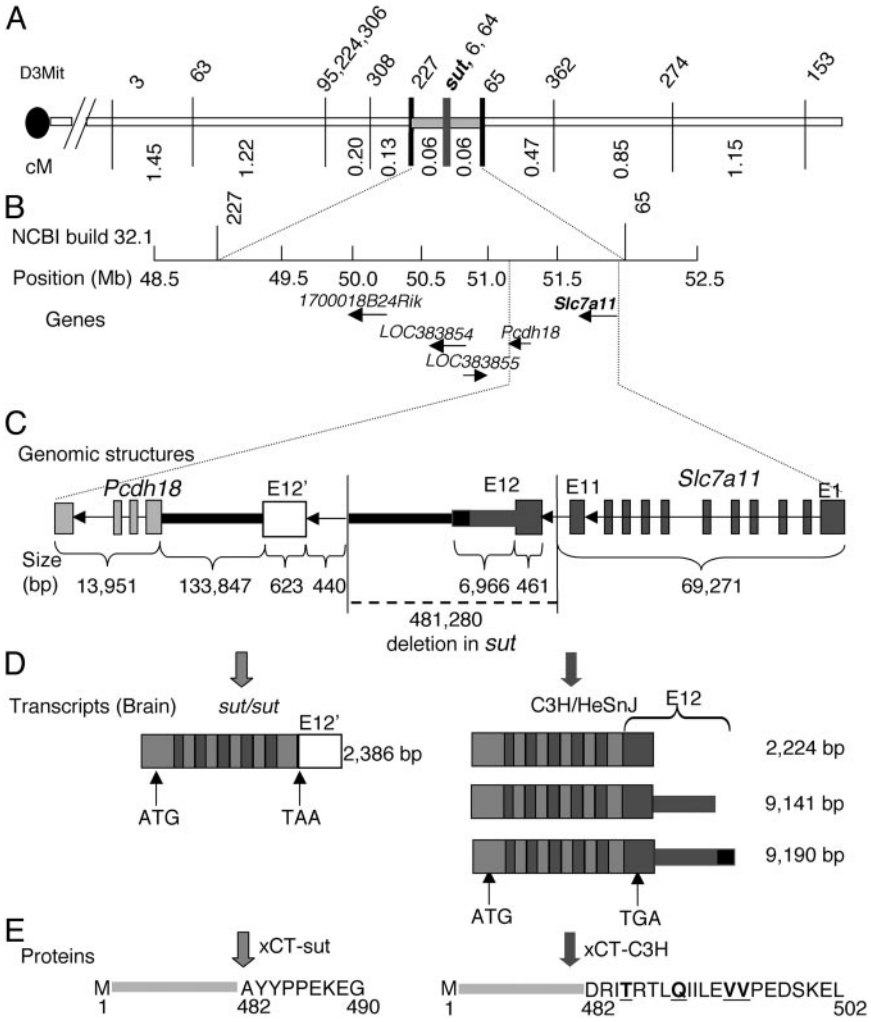

Fig. 1. Positional cloning of the *sut* gene. (*A*) High-resolution genetic map of the *sut* genetic interval. (*B*) High-resolution physical map. Five known genes in the *sut* interval are listed with arrowheads indicating transcriptional orientations. (*C*) Region of the genome between *Pcdh18* and *Slc7a11*. The *sut* deletion is depicted by a dashed line. The sizes of introns (lines), exons (boxes), and intergenic regions (bold lines) are given in base pairs. (*D*) Transcripts of *Slc7a11* in *sut* and control (C3H/HeSnJ) brains identified by 3′ RACE. In *sut*, *Slc7a11* utilizes an alternative exon 12 (E12′, open box) from beyond the deleted region. (*E*) Predicted proteins, xCT-*sut*, and xCT-C3H, encoded by transcripts in *sut* and C3H/HeSnJ respectively. The underlined bold residues in xCT-C3H are conserved across species.

Rescue of *sut* Phenotype in Transgenic Mice. We injected bacterial artificial chromosome (BAC) RP23-2203 into pronuclei derived from hybrid (C3H/HeRos × C57BL/10 Rospd) F_2 females. BAC-positive pups were mated with C3H/HeSnJ *sut*/*sut* mice to produce F_1 progeny. BAC-positive F_1 pups were backcrossed to *sut*/*sut* to produce F_2 progeny. Each F_2 pup was typed for coat color, presence of the BAC transgene, and presence of the deletion in the *Slc7a11* gene.

Transport of [^{35}S]Cystine and [^{3}H]Leucine. Melanocytes from wild type (melan-a) and mutant (*sut*/*sut*) mice grown with βME were rinsed three times in warm PBSG [10 mM sodium phosphate/137 mM NaCl/3 mM KCl (pH 7.4), containing 0.9 mM $CaCl_2$, 0.49 mM $MgCl_2 \cdot 6H_2O$, and 5.6 mM glucose], and then incubated in uptake medium {PBSG containing [^{35}S]cystine (10 μCi/0.5 ml; 1 Ci = 37 GBq), [^{3}H]leucine (1 μCi/0.5 ml), and unlabeled leucine (10 μM), with or without βME}. The specificity of the cystine uptake system was determined in the presence of 2.5 mM unlabeled glutamic acid and arginine, separately.

Melanin Analysis. Eumelanin and pheomelanin were quantitatively analyzed (14) by HPLC based on the formation of pyrrole-2,3,5-tricarboxylic acid (PTCA) by permanganate oxidation of eumelanin and 4-amino-3-hydroxyphenylalanine (4-AHP) by hydriodic acid reductive hydrolysis of pheomelanin, respectively (1). These specific degradation products were determined by HPLC.

HPLC Analysis of Glutathione. Glutathione was analyzed by HPLC (15). To correct for the artifactual oxidation of glutathione, an aliquot of each sample was treated with 5% perchloric acid in the presence of 50 mM *N*-ethylmaleimide and analyzed in parallel.

Screening of HPS Patients. RNA was isolated from cultured fibroblasts of 15 HPS patients enrolled in a protocol approved by the National Institute of Child Health and Human Development and the National Human Genome Research Institute institutional review boards to study the clinical and molecular aspects of HPS. Mutation analysis for human *SLC7A11* was performed on each patient's cDNA (transcribed from RNA by using Superscript RT-PCR, Invitrogen) in three overlapping fragments by using standard PCR and sequencing conditions.

PCR products spanning the 13 exons of *SLC7A11* plus the adjacent intron and noncoding sequences were screened in

genomic DNA of 17 other HPS patients who likewise lack mutations in the known human HPS genes plus one normal Caucasian control.

Dopa Histochemistry and Electron Microscopy. Dopa (3,4-dihydroxyphenylalanine) histochemistry was carried out by a method modified from that of Boissy *et al.* (16). Cells were fixed in 2.5% glutaraldehyde/2% paraformaldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 1 h at room temperature and washed before incubation in L- or D-dopa (0.1% in cacodylate buffer) for two 2.5 h intervals at 37°C. (D-dopa staining was used as a control and produced no stain.) The cells were washed as before and postfixed in 1% osmium tetroxide with 1.5% potassium ferrocyanide in cacodylate buffer for 1 h at room temperature. Three final washes were carried out before dehydration and embedding for sectioning.

Results

Positional Cloning of the *sut* Gene. To obtain a high-resolution genetic map of the *sut* gene region on mouse chromosome 3, 1,474 progeny of an interspecific backcross were typed for coat color (the *sut* gene) and numerous microsatellite markers. This backcross defined a 0.12 cM genetic interval (Fig. 1*A*), consisting of 3 megabases of DNA containing five predicted genes (Fig. 1*B*). One gene, *Slc7a11*, which encodes the xCT cystine/glutamate exchanger (17), produced no product on amplification of *sut* cDNA by RT-PCR using primers from exons 1 and 12. Sequencing revealed a large deletion (481,280 bp) extending from intron 11 through exon 12 and into the *sut* intergenic region adjacent to the *Pcdh18* gene (Fig. 1*C*), creating a new splice site and replacement of exon 12 with exon 12′ (E12′, Fig. 1 *C* and *D*). 3′ RACE revealed a new stop codon in exon 12′ and predicted a modified and truncated protein carboxyl terminus (Fig. 1*E*).

Deficiency of Expression of *Slc7a11* in *sut* Mice and Its Effect on Pigmentation in *sut* and Transgenic Mice. Northern blot analyses of poly(A)-RNA from normal and *sut*-mutant brain and melanocytes demonstrated a marked deficiency of the 9-kb (brain) (18) and 9.5-kb (melanocyte) *Slc7a11* mRNA transcripts in *sut/sut* (Fig. 2*A*). We confirmed the identification of *sut* as *Slc7a11* by rescuing the *sut*-mutant phenotype by inserting wild-type *Slc7a11* into the genome of *sut*-mutant transgenic mice. BAC RP23-22O3, which exclusively contains the *Slc7a11* gene, restored normal agouti pigmentation to *sut* mutants (Fig. 2*B*), demonstrating *Slc7a11* is critical for pheomelanin production and is the bona fide *sut* gene.

To provide clear visualization of the role of the *Slc7a11* gene in pheomelanin production, we transferred the *sut* allele to a stock containing the semidominant "yellow" (A^y) allele, which is conspicuously yellow because of constitutive expression of the agouti locus (19). Their intense pheomelanic pigmentation was muted to a light cream color when the mouse was also homozygous for *sut* (Fig. 2*C*), indicating that loss of expression of *Slc7a11* causes marked inhibition of pheomelanogenesis.

Control of Pheomelanin Levels by *Slc7a11*. To quantify the effect of lost *Slc7a11* expression on pheomelanin production, we analyzed eumelanin and pheomelanin by HPLC in hair of C3H +/+, *sut*/+, and *sut/sut* mice. The agouti (*A/A*) genotype of these mice produces both eumelanin and pheomelanin in hair. Hair from *sut* mutants contained only 40% normal pheomelanin levels with no significant effect on eumelanin, and normal pheomelanin levels were restored in *sut* mice transgenic for the wild-type *Slc7a11* gene (Table 1). The effect of the *Slc7a11 sut* mutation on pheomelanin production was markedly accentuated on the A^y/a background, reducing pheomelanin levels to <20% of the control level. Also, the low level of eumelanin was increased 4-fold on loss of *Slc7a11* expression. We likewise observed a large reduction in pheomelanin in cultured *sut* melanocytes (Table 2), which also exhibited an increase in eumelanin, making them among the most eumelanic of cultured melanocytes thus far examined (20). Combined genetic, transgenic rescue, and biochemical data therefore provide compelling evidence that the *Slc7a11* xCT transporter is critical for the production of pheomelanin.

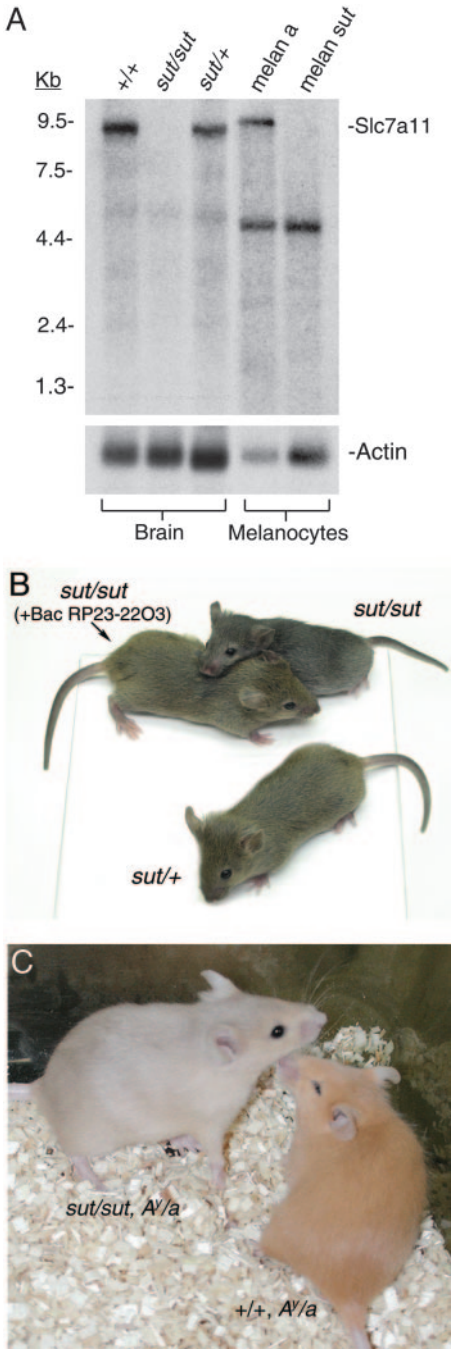

Fig. 2. Altered regulation of *Slc7a11* in *sut* mutants. (*A*) Northern blots of poly(A)-mRNA were probed with labeled *Slc7a11* (*Upper*) and β-actin (*Lower*) cDNAs. (*B*) One transgenic rescued *sut/sut* agouti pup (positive for BAC RP23-22O3, which contains *Slc7a11*) plus a *sut/sut* littermate and a heterozygous (*sut*/+) control. Note agouti color in BAC-positive pup compared with the gray color of *sut/sut* pup. (*C*) There is a near-complete loss of yellow/red (pheomelanin) pigment in *sut/sut*, A^y/a mutants compared with the +/+, A^y/a controls.

Table 1. Melanin analyses of hair

Mice	Pheomelanin, μg/mg	Eumelanin, μg/mg	Pheomelanin/ eumelanin
C3H +/+	3.80 ± 0.398	53.6 ± 5.18	0.073
C3H *sut*/+	3.77 ± 0.302	75.7 ± 3.10	0.051
C3H *sut*/*sut*	1.55 ± 0.212*	75.9 ± 5.01	0.033
C3H *sut*/*sut* + BAC RP23–2203	4.77 ± 1.00	62.9 ± 2.33	0.077
B6 A^y/*a* +/+	40.5 ± 2.11	0.523 ± 0.127	92.2
B6 A^y/*a*, *sut*/*sut*	7.27 ± 0.908**	2.138 ± 0.418*	4.4

Values are mean ± SEM of 3 or 4 samples. *, $P \leq 0.05$; **, $P < 0.001$ compared with +/+ mice of the same strain.

***Slc7a11* Mutation Reduces Cystine Transport into Melanocytes and Other Cells.** A possible explanation for pheomelanin deficiency in *sut* mice is that loss of *Slc7a11* reduces transport of cystine into melanocytes. Indeed, transport of radiolabeled cystine into *sut*-mutant melanocytes was depressed to 20–30% of control rates (Fig. 3*A*). Similarly decreased rates of cystine transport were observed in *sut*-mutant macrophages. The effect of the *Slc7a11* mutation is specific because there was no diminution in uptake of [^{3}H]leucine (Fig. 3*A*) or [^{35}S]cysteine (data not shown). Inhibition of transport of [^{35}S]cystine into wild-type (melan-a) melanocytes by glutamate, but not arginine (Fig. 3*A*), confirms that these measurements are specific for the *Slc7a11* xCT transporter (21).

Transport of Cystine by the xCT Transporter Is Necessary for Survival and Proliferation of Cells Under Normal Culture Conditions. Sulfhydryl amino acids exist almost entirely in their oxidized form under normal culture conditions (17, 21). We hypothesized therefore that viability of cultured *sut* cells would be adversely affected, because they likely do not transport cystine intracellularly at a rate sufficient for survival. In fact, *sut* melanocytes grew very poorly under normal culture conditions, and growth was improved, to essentially normal rates, by supplementation of the medium with a sulfhydryl reducing agent, βME (Fig. 3*B*). Without βME, these cells featured aberrant dendritic morphology, and >90% were nonviable by trypan blue staining after 4 days in culture. It seems likely that βME improves growth by preventing oxidation of cysteine, which is transported at normal rates (see *Slc7a11 Mutation Reduces Cystine Transport into Melanocytes and Other Cells*) in *sut* cells by alternative systems such as the alanine–serine–cysteine transporter (17). We observed similar reductions in cell proliferation and survival under normal oxidizing conditions and restoration to normal rates under reducing conditions, for *sut* fibroblasts (Fig. 3*B*). Further, we observed an intermediate growth rate of *sut*/+ fibroblasts

Table 2. Melanin analyses of cultured melanocytes

Melanocytes	Pheomelanin, μg/mg of protein	Eumelanin, μg/mg of protein	Pheomelanin/ eumelanin
Plus βME			
Melan-a	9.14 ± 0.47	88.9 ± 18.5	0.103
sut	0.80 ± 0.08**	404 ± 29.9**	0.0020
Minus βME			
Melan-a	22.6 ± 2.3	196 ± 25	0.115
sut	1.67 ± 0.44**	990 ± 289**	0.0017

Melan-a and *sut* melanocytes were cultured with 100 μM βME until they reached 80–90% confluency. Thereupon, cells were split and cultured in the presence (plus βME) or absence (minus βME) of βME for 1 day before analysis. Values are mean ± SEM of 3 or 4 samples. **, $P \leq 0.001$ compared with melan-a mice.

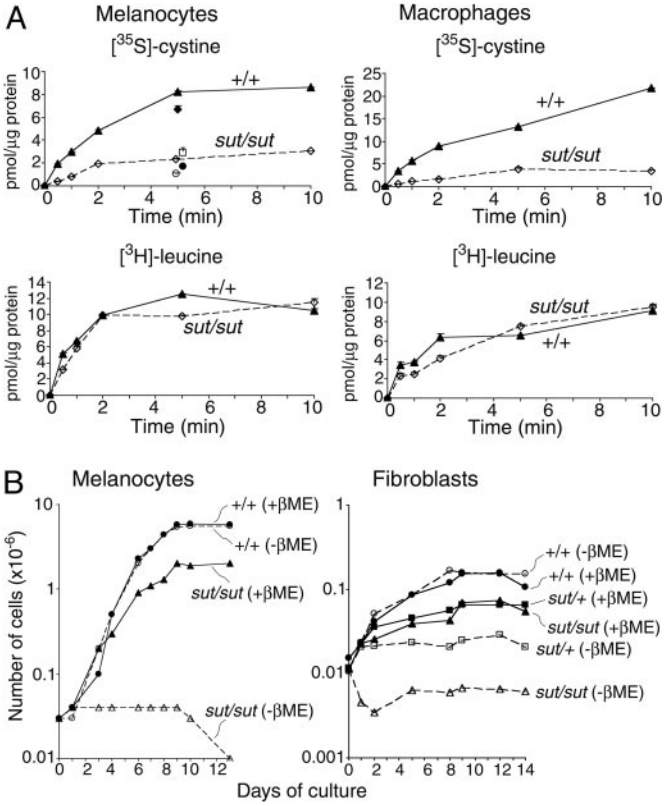

Fig. 3. Cystine transport and growth are reduced in cultured *sut* cells. (*A Upper*) [^{35}S]Cystine transport was measured in melanocytes and macrophages from +/+ (▲) and *sut*/*sut* (◇) mice cultured under normal oxidizing conditions. Effects of 2.5 mM glutamic acid [melan-a +/+ melanocytes (●); sut-melanocytes (□)] and 2.5 mM arginine [melan-a +/+ melanocytes (◆); sut-melanocytes (○)] on the transport of [^{35}S]cystine were assayed at 5 min. (*A Lower*) [^{3}H]Leucine transport into melanocytes and macrophages. Values are the average of duplicates from a representative experiment. (*B*) Growth of *sut* cells is attenuated under oxidizing culture conditions. Fibroblasts and melanocytes were grown with (+) and without (−) βME. Values are the average of duplicates from a representative experiment.

minus βME, indicating that reduced xCT protein produced in heterozygous cells is limiting for cystine uptake and utilization.

The xCT Transporter Maintains Normal Glutathione Levels. Cysteine is a component of the tripeptide glutathione, which is critically important in the control of harmful reactive oxygen species. No glutathione was detectable in *sut* melanocytes grown without βME (Fig. 4). Glutathione in *sut* melanocytes cultured with βME was measurable but lower than in wild-type melanocytes. These results emphasize the importance of the xCT transporter in maintaining normal glutathione concentration and provide a rationale for the loss of viability of *sut* cells cultured under normal oxidizing conditions.

***Slc7a11* Regulates Tyrosinase Transport in Melanocytes.** The morphology and melanosome density of cultured immortalized *sut* melanocytes appeared similar to those of control melanocytes by light microscopy (Fig. 5), although clusters of melanosomes were more prevalent on ultrastructural analysis than in control cells (data not shown). Dopa-staining indicated an abnormal accumulation of the enzyme tyrosinase in tubular perinuclear structures, possibly the trans-Golgi network (Fig. 6*A*). This accumulation was not seen in wild-type cells or in *sut* cells grown with βME (Fig. 6*B*), which had a normal distribution of dopa stain in transport vesicles and melanosomes. Hence, at least a portion of

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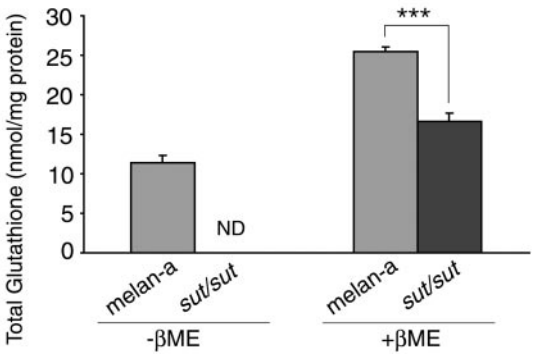

Fig. 4. Loss of *Slc7a11* expression causes loss of glutathione. Total glutathione was measured in five separate analyses of melan-a and *sut* melanocytes grown in the absence (−βME) or presence (+βME) of βME (See Table 2 legend for details). Total glutathione was >93% the reduced GSH form in all analyses. Values are mean ± SEM. ***, $P \leq 0.001$. ND, not detected.

this melanosomal enzyme undergoes abnormal trafficking in *sut* cells grown without βME.

Screening for *SLC7A11* Mutations in HPS Patients. In the mouse, *sut* is 1 of at least 16 pigmentation mutants that provide models for human HPS (8). Accordingly, we screened for human *SLC7A11* (gI no. 18141306) mutations in 32 patients with features of HPS who lack mutations in the seven known human HPS loci. Although a number of nonpathological SNPs were observed, no deleterious mutations were found.

Discussion

A current model suggests that the eumelanin/pheomelanin ratio in mammalian pigmentation is controlled solely and indirectly by modulation of the activity of tyrosinase, the rate-limiting enzyme for melanin synthesis (22). This model postulates that at low tyrosinase concentrations dopaquinone reacts in melanosomes with sulfhydryls such as cysteine, yielding cysteinyl-dopa (1), and increased quantities of pheomelanin are produced. Although useful, this model for control of the eumelanin/pheomelanin ratio is incomplete. Our data demonstrate that the xCT transporter is a critical player in the control of pigmentation. However, unlike tyrosinase, it directly affects pheomelanin production with small increases in eumelanin in hair and cultured *sut* melanocytes. The loss of yellow pigment in *sut* mutants indicates that a critical rate of transport of cystine into melanocytes is essential for pheomelanin synthesis *in vivo*. The *Slc7a11* gene directly affects this pheomelanin synthesis pathway. These results are consistent with biochemical evidence that cysteine is an important component of pheomelanin (1).

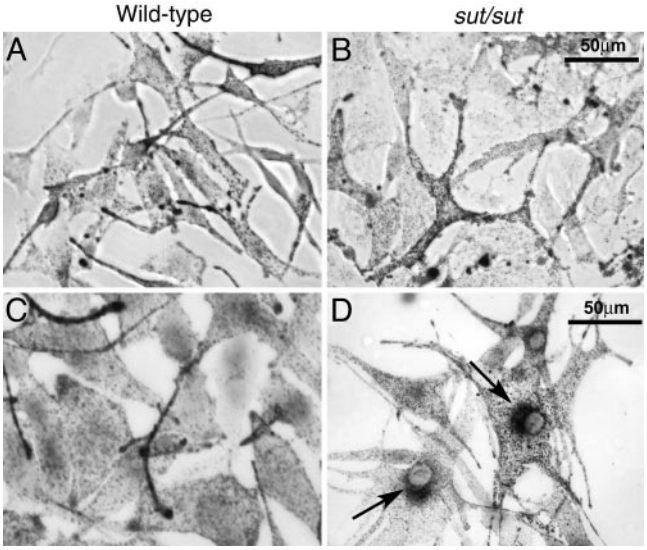

Fig. 5. Microscopy of wild-type (melan-a, *A* and *C*) and *sut*-mutant (*B* and *D*) melanocytes. (*A* and *B*) Bright-field images. (*C* and *D*) Dopa-stained cells; arrows in *D* indicate the perinuclear distribution of dopa reaction product in *sut* cells.

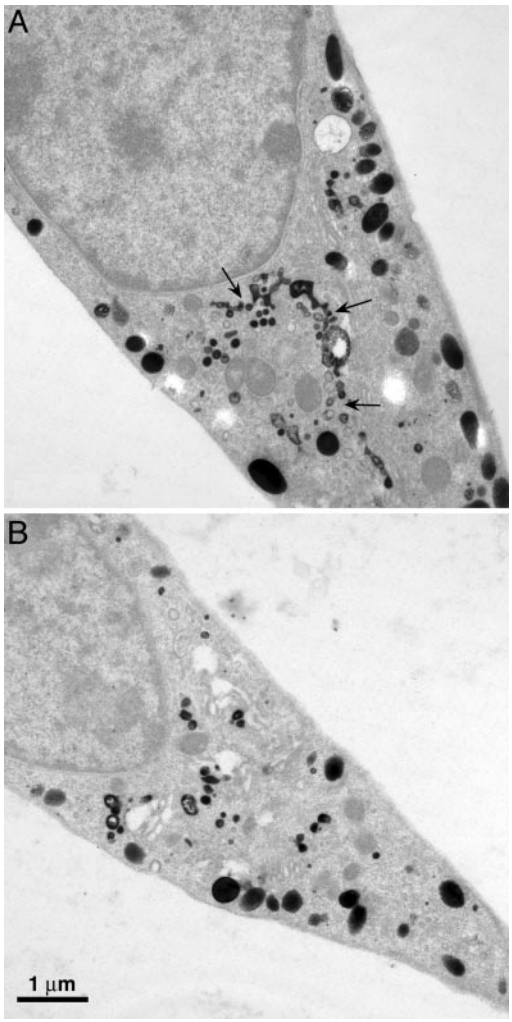

Fig. 6. Ultrastructure of L-dopa-stained *sut* melanocytes. Melanocytes from *sut* mice were cultured in the absence (*A*) and presence (*B*) of βME, stained with dopa, and observed in the electron microscope. Arrows in *A* indicate increased staining of tubular/vesicle structures, possibly the trans-Golgi network, typically observed in *sut* cells cultured without βME.

Several genes [melanocortin 1 receptor (*Mc1r*), pro-opiomelanocortin α (*Pomc1*), agouti (*a*), attractin (*Atrn*), and mahogunin (*Mgrn1*)] regulate the switching between eumelanin and pheomelanin synthesis in mouse hairshafts (23, 24). A knockout of the γ-glutamyl transpeptidase gene (25) and a mutation of the gray-lethal (*ostm1*) gene (26), which encodes a unique transmembrane protein (27), apparently affect pheomelanin. Loss of the former gene indirectly lowers tissue cysteine levels and produces a gray coat; mutation of the latter causes clumping of pheomelanin granules and a gray coat. However, hair pheomelanin concentrations were not chemically ascertained in either case.

It is formally possible that the role of xCT, similarly to the agouti or melanocyte stimulating hormone proteins, is to signal

pigment type switching. Alternatively, xCT may act in a permissive role to supply enough cystine so that pheomelanin synthesis can proceed. In this case, *sut* mutant melanocytes would be expected to divert dopaquinone that would otherwise have been converted to cysteinyl-dopa into the eumelanin branch. The 4-fold increase in eumelanin in hair of A^y/a, *sut*/*sut* mutants is consistent with such a role. The permissive role likewise seems more plausible given the well documented role of xCT in cystine transport. However, additional experiments such as the analysis of expression of *Slc7a11* mRNA during agouti banding are required to unequivocally distinguish these possible roles.

sut cells do not proliferate or survive under normal oxidizing culture conditions. Our data demonstrate that the xCT transporter maintains normal rates of delivery of cystine into cultured cells and thus is indispensable for cell growth and survival. Reduced cell survival probably results from loss of critical cellular defenses against reactive oxygen species by glutathione, which is substantially lost in *sut* melanocytes, particularly under oxidizing conditions in which cystine transport is greatly depressed. These results are consistent with studies showing that *Slc7a11* expression is elevated in cells that require high glutathione synthesis for antioxidant defense (17). *sut* cells thus provide a model for oxidative stress-related diseases and their therapies. We speculate that alleles of *SLC7A11* may regulate variation in human pigmentation (28, 29) as well as susceptibility to skin cancer and other harmful effects of UV radiation (2).

Whereas *Slc7a11*-deficient cells in culture rapidly expire because of oxidative stress, *sut* mice appear healthy. A likely explanation is that plasma, in contrast to typical culture media, contains significant levels of cysteine (30). The widely expressed alanine–serine–cysteine transporter (17) would be expected to transport plasma cysteine intracellularly at levels sufficient for viability of *sut* tissues. Nevertheless, cells such as melanocytes, with attendant high cysteine requirements, manifest a mutant phenotype in *sut* mice.

The oxidizing environment generated by culturing *Slc7a11*-negative *sut* melanocytes in the absence of βME causes abnormal trafficking of the critical melanosomal enzyme tyrosinase to a perinuclear (perhaps trans-Golgi) location, although this abnormality does not alter production of eumelanin pigment. Abnormal trafficking of tyrosinase is apparently a general feature of HPS, being observed in the reduced-pigmentation HPS mouse mutant (10) and in melanocytes of HPS-1, HPS-2, and HPS-3 patients (31, 32). Whether Slc7a11 directly or indirectly regulates trafficking of tyrosinase and other important components of lysosome-related organelles requires further investigation.

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